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 Washington, D.C. 20231

**Application Transmittal Letter**

Dear Sir:

Transmitted herewith for filing is the patent application of: Joseph D. Mosca and Kevin R. McIntosh. entitled:

**MESENCHYMAL STEM CELLS AS IMMUNOSUPPRESSANTS**

Enclosed with the application are:

1. Specification ( 13 sheets);
2. Claims ( 2 sheets);
3. Abstract ( 1 sheet);
4. Drawings (informal; 4 sheets);
5. Unexecuted Declaration of Joseph D. Mosca and Kevin R. McIntosh;
6. Verified Statement Claiming Small Entity Status;
7. Our check (No. 28700 ) in the amount of \$419.00; and
8. Self-addressed, postage paid, return receipt postcard.

The fee has been calculated as follows:

|                    | Number of<br>Claims Filed | Extra<br>Claims | Rate        | Fee             |
|--------------------|---------------------------|-----------------|-------------|-----------------|
| Basic Fee          |                           |                 | \$380.00    | \$380.00        |
| Total Claims       | 16 - 20                   | 0               | x \$9.00 =  |                 |
| Independent Claims | 4 - 3                     | 1               | x \$39.00 = | \$39.00         |
| <b>TOTAL</b>       |                           |                 |             | <b>\$419.00</b> |

The Commissioner is authorized to charge payment of any additional filing fees required under 37 CFR 1.16 associated with this communication or credit any overpayment to Deposit Account No. 03-0678. A duplicate copy of this paper is enclosed.

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Assistant Commissioner for Patents  
 Washington, DC 20231

*Raina Semionow*  
 Raina Semionow

March 12, 1999  
 Date

Respectfully submitted,

*Raina Semionow*

Raina Semionow  
 Reg. No. 39,022

CARELLA, BYRNE, BAIN, GILFILLAN,  
 CECCHI, STEWART & OLSTEIN  
 6 Becker Farm Road  
 Roseland, NJ 07068  
 Tel. No.: (973) 994-1700  
 Fax No.: (973) 994-1744

**VERIFIED STATEMENT(DECLARATION) CLAIMING SMALL ENTITY STATUS**

**37 CFR 1.9(f) and 1.27(c) - SMALL BUSINESS CONCERN**

I hereby declare that I am

☐

the owner of the small business concern identified below:

☒

an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF CONCERN: Osiris Therapeutics, Inc.

ADDRESS OF CONCERN: 2001 Aliceanna Street, Baltimore, MD 21231

I hereby declare that the above identified small business concern qualifies as a small business concern as defined in 13 CFR 121.3-18, and reproduced in 37 CFR 1.9(d), for purposes of paying reduced fees under Section 41(a) and (b) of Title 35, United States Code, in that the number of employees of the concern, including those of its affiliates, does not exceed 500 persons. For purposes of this statement, (1) the number of employees of the business concern is the average over the previous fiscal year of the concern of the number of persons employed on a full-time, part-time or temporary basis during each of the pay periods of the fiscal year, and (2) concerns are affiliates of each other when either, directly or indirectly, one concern controls or has the power to control the other, or a third-party or parties controls or has the power to control both.

I hereby declare that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention entitled:

**MESENCHYMAL STEM CELLS AS IMMUNOSUPPRESSANTS**

Inventors: Joseph D. Mosca and Kevin R. McIntosh

described in:

☒

the specification filed herewith

☐

application serial no. \_\_\_\_\_, filed \_\_\_\_\_

☐

patent no. \_\_\_\_\_, issued \_\_\_\_\_

If the rights held by the small business concern are not exclusive, each individual, concern or organization having rights to the invention is listed below\* and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d) or as a nonprofit organization under 37 CFR 1.9(e).

\*NOTE: Separate verified statement are required from each named person, concern or organization having rights to the invention averring to their status as small entities. (37 CFR 1.27)

NAME: \_\_\_\_\_

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I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate. (37 CFR 1.28(b)).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

NAME OF PERSON SIGNING: James S. Burns

TITLE IN ORGANIZATION President and CEO

ADDRESS OF PERSON SIGNING 2001 Aliceanna Street, Baltimore, MD 21231

SIGNATURE 

DATE

3.11.99

**CARELLA, BYRNE BAIN, GILFILLAN, CECCHI, STEWART & OLSTEIN**  
6 Becker Farm Road - Roseland, NJ 07068 - (973) 994-1700

Docket No 640100-295  
#22458 v1 - SES-Osiris-295

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## MESENCHYMAL STEM CELLS AS IMMUNOSUPPRESSANTS

This application is based on and claims priority of U.S. provisional application serial no. 60/080,678 filed April 3, 1998.

15

The present invention relates to the field of inducing immunosuppression by using mesenchymal stem cells to inhibit T cell activation against antigens.

### Background of the Invention

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In a normal immune response, T cells are activated by a first, antigen specific, signal which stimulates T cells via the T cell antigen receptor and confers antigen specificity to the immune response; and a second, costimulatory, signal delivered by costimulatory molecules found on the surface of antigen presenting cells. Both signals are required to induce T cell proliferation. If the second costimulatory signal does not occur, or if the co-stimulation pathway is blocked or downregulated, activation of T cells will be reduced or eliminated. This results in hyporesponsiveness of the T cells to antigen and can induce a state of T cell anergy, or T cell nonresponsiveness, wherein the T cells will not proliferate when presented with antigen.

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The best characterized co-stimulatory molecules on antigen-presenting cells are the structurally related glycoproteins B7-1 (CD80) and B7-2 (CD86). These are homodimeric members of the immunoglobulin superfamily found exclusively on the surface of cells capable of stimulating T-cell growth. The receptor for B7 molecules on the T-cell is CD28, another

member of the immunoglobulin superfamily. Ligation of CD28 by B7-1 or B7-2 or by anti-CD28 antibodies will co-stimulate the growth of naive T-cells, while antibodies to the B7 molecules, which inhibit B7 binding to CD28, inhibit T-cell responses.

5       The function of the immune system is to eliminate foreign cells that may contain pathogens, while maintaining unresponsiveness or tolerance against self-antigens. Tolerance is manifested by T cell anergy, characterized by the survival, but nonresponsiveness of T cells. However, the immune system may attack self-constituents, causing autoimmune disease. Autoimmune diseases are believed to originate in the abnormal immune response to self-antigens,  
10 either due to a change in self-antigens or exposure to crossreactive antigens. Autoimmune diseases caused by antibody-mediated immune responses to self-antigens include diseases such as rheumatoid arthritis, systemic lupus erythematosus and inflammatory bowel disease.

15       It is desirable to improve present treatments of autoimmune disease or other undesirable immune reactions which use general immunosuppressive agents such as corticosteroids, azathioprine, or cyclosporine A. These treatments are nonselective and do not distinguish between normal and abnormal immune responses. These drugs often have adverse side effects, including general suppression of the immune system with risks of infection and neoplasia, as well as the development of diseases such as diabetes, osteoporosis, leukopenia and hypertension.

20       Accordingly, in certain circumstances, such as autoimmune disease, a particular immune response may not be desired. Alternative approaches for treatment of these conditions are needed for patients who cannot withstand, or do not respond to, conventional chronic, non-specific drug therapy.

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### Summary of the Invention

It has been discovered that human mesenchymal stem cells can be used to deliver antigens to the immune system such that an immune response to the antigen will be inhibited, i.e. eliminated, reduced or ameliorated. The reduction or elimination of an immune response by mesenchymal stem cells as described herein can be used as a method to ameliorate an immune response in a recipient against autoimmune disease.

Accordingly the methods of the present invention are particularly useful for eliminating, reducing or ameliorating unwanted or abnormal T cell immune responses. In one aspect the method involves administering to an animal mesenchymal stem cells which have been modified to carry an antigen. Presentation of the antigen to the T cell in the absence of a costimulatory signal induces an antigen-specific state of hyporesponsiveness, or even nonresponsiveness or anergy in the T cell to subsequent challenge to the T cell by the antigen. Thus, an immune response is reduced or eliminated. The elimination, reduction or amelioration of an immune response as described herein can be used as a method to treat or inhibit an unwanted or abnormal immune response such as occurs in autoimmune disease.

In another aspect of the present invention mesenchymal stem cells can be used to present to the immune system molecules that block costimulation pathways which are required to effect an immune response. The elimination, reduction or amelioration of an immune response as described herein can be used as a method to treat or inhibit an unwanted or abnormal immune response such as occurs in autoimmune disease. Blocking the costimulatory signal inhibits T cell activation by antigen specific signals presented by antigen presenting cells that may be in the proximity of the T cells.

In a still further aspect of the invention, the mesenchymal stem cells are used to deliver to the T cells an antigen and a molecule that inhibits the delivery of a costimulation signal to the T cells. Delivering a specific antigen to target T cells in conjunction with blocking the costimulatory signal inhibits specific T cell activation in the presence of antigen specific signal presented by the antigen-presenting mesenchymal stem cell. In a preferred embodiment,

molecules that block T cell costimulation are selected from cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or CTLA-4Ig, a fusion protein of CTLA-4 and the constant region of human IgG-1.

### Brief Description of the Drawings

Fig. 1 shows a reduction in the proliferative response of tetanus toxoid specific T cells to restimulation using peripheral blood mononuclear cells as antigen presenting cells after the T-cells were co-cultured with mesenchymal stem cells that were pulsed with tetanus toxoid.

Fig. 2. Flow cytometry analyses of human mesenchymal stem cells confirming the transduction of the cells with CTLA-4 (membrane bound) (Fig. 2A) and soluble form CTLA4-Ig (Fig. 2B).

Fig. 3. Inhibition of T cell stimulation by human mesenchymal stem cells transduced with CTLA-4.

Fig. 4. Inhibition of T cell stimulation by human mesenchymal stem cells transduced with CTLA4-Ig.

### Detailed Description of Preferred Embodiments

This invention relates to methods for reducing, inhibiting or ameliorating an immune response to a specific antigen, *in vivo*, by employing mesenchymal stem cells as antigen presenting cells to present antigen to the animal. The mesenchymal stem cells ameliorate, inhibit or reduce the T cell response to the antigen because the mesenchymal stem cells do not produce, or do not produce sufficient amounts of, the costimulatory signal required to activate the T cells. Instead, the mesenchymal stem cells induce hyporesponsiveness of a targeted T cell population to antigen, i.e., there is an ameliorated, inhibited or reduced T cell immune response to the antigen upon subsequent presentation of the antigen to the T cell.

Accordingly, the mesenchymal stem cells can be modified to present antigen to a target T cell population comprising T cells having receptors for the specific antigen. In one embodiment, the mesenchymal stem cells are modified by contact with antigen *in vitro*. In another embodiment, the mesenchymal stem cells are genetically manipulated to express an antigenic molecule. The mesenchymal cells are preferably autologous to the recipient of the mesenchymal stem cells.

As used herein, the term antigen is intended to include a protein, polypeptide, lipid, glycoprotein, etc. In one aspect of the invention, mesenchymal stem cells are modified to have at least one exogenous antigen fragment bound to a primary surface molecule of the cell such that at least one antigen fragment is presented to inhibit or reduce the proliferative response of lymphocytes. In one embodiment, the mesenchymal stem cell is contacted with at least one antigen (antigen-pulsing). The mesenchymal stem cell processes the antigen into an antigen fragment for presentation by the mesenchymal stem cells. In another embodiment, the mesenchymal stem cell is genetically manipulated to express an antigenic molecule.

In another aspect of the invention, it is contemplated that the mesenchymal stem cells are modified to express a specific autoantigen, particularly an autoantigen that mediates the immune response in autoimmune diseases.

The present invention further relates to a method of inhibiting, ameliorating or reducing a T cell response to a specific antigen by administering, *in vivo*, mesenchymal stem cells used as antigen presenting cells, with the mesenchymal stem cells being further modified with a molecule that blocks the delivery of a T cell costimulation signal to the targeted T cell. The target T cell population may or may not have already been presented once with antigen. Thus, in addition to inducing hyporesponsiveness of the T cells to challenge by the antigen, the mesenchymal stem cells further ameliorate, inhibit or reduce the T cell immune response by blocking costimulatory signals to the T cell that may be provided by an antigen presenting cell. This treatment inhibits full activation of the T cells and results in hyporesponsiveness or even nonresponsiveness of the T cells.

With respect to this aspect of the invention, the antigen presenting cells that provide costimulatory signals to the T cells can be endogenous antigen presenting cells or may be the antigen-presenting mesenchymal stem cell. In the case where mesenchymal stem cells which may express the costimulatory signal required to activate T cells are used, these mesenchymal stem cells will be further modified with a molecule that blocks delivery of the costimulatory signal.

In accordance with this aspect of the invention, it is contemplated that the mesenchymal stem cells are modified to express a specific autoantigen, particularly an autoantigen that mediates the immune response in autoimmune diseases.

According to the method of the present invention, a molecule that inhibits the delivery of a costimulatory signal to a T cell is one that interferes with the T cell costimulation pathway such that the T cells do not proliferate against antigen as occurs in a normal immune response. As the term is used herein, a T cell costimulation signal is delivered when a costimulatory molecule interacts with its T cell receptor. Accordingly, in a preferred embodiment, the method involves the expression of a molecule by the mesenchymal stem cells which will block or interfere with such interaction. By blocking this interaction on primed T cells (i.e., T cells already presented with the first antigen specific signal), full activation of the T cells by antigen presenting cells is inhibited. The T cells exhibit hyporesponsiveness or nonresponsiveness to antigen, which results in an amelioration, reduction or elimination of an immune response by the T cells against antigen.

In a preferred embodiment of this aspect of the invention, the costimulatory molecules are B7-1 and B7-2 and the T cell receptor is CD28. In accordance with this embodiment, the mesenchymal stem cell is preferably genetically engineered to express CTLA-4 or CTLA4-Ig. Both CTLA-4 and CTLA4-Ig interfere with the interaction of B7 and CD28, thus inhibiting the delivery of the costimulatory signal by the antigen to the T cell.



Activated T-cells express the receptor CTLA-4, in addition to CD28, which binds B7 molecules with a higher affinity than does CD28. CTLA-4 closely resembles CD28 in sequence, and the two molecules are encoded by closely linked genes. CTLA-4 is a competitive antagonist of CD 28. CTLA-4 inhibits the delivery of costimulatory signal, by either outcompeting CD28  
5 for binding to B7 or downregulating the signal, resulting in the inhibition of full activation, and therefore proliferation of the T-cell expressing it.

A soluble form of CTLA-4 is the fusion protein CTLA4-Ig. The CTLA-4 molecule is incorporated as part of a fusion protein that contains a region that increases the stability and  
10 solubility of the molecule. Blocking of T cell activation by CTLA4-Ig, the B7 specific fusion protein, has also been described (Kirk, et. al., PNAS 1997).

In a still further aspect, the method of the present invention reduces, inhibits or ameliorates an immune response to an antigen, *in vivo*, by employing mesenchymal stem cells as  
15 a vehicle to present to the immune system a molecule that blocks T cell costimulation. The mesenchymal stem cells induce hyporesponsiveness or nonresponsiveness of the T cell to an antigen by interference with the delivery of a costimulatory signal to the T cell. This results in a reduction, amelioration or elimination of the T cell immune response to an antigen after presentation of the antigen to the T cell.

Accordingly, the mesenchymal stem cells can be modified to present to the immune  
20 system a molecule that blocks or interferes with delivery of a costimulatory signal to a T cell that may already be primed with an antigen specific signal from an endogenous antigen presenting cell. The mesenchymal stem cells are preferably modified to express CTLA-4 or CTLA4-Ig. In  
25 one embodiment, the mesenchymal stem cells are genetically manipulated to express the blocking molecules prior to contact with the T cells. In accordance with this aspect, after the mesenchymal stem cells are administered, the method results in blocking of costimulation of T cells by antigen presenting cells and, thus, a subsequent reduced T cell immune response is obtained.

In a preferred embodiment of this aspect of the invention, mesenchymal stem cells are used as a platform to express cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) or CTLA-4Ig, a fusion protein of CTLA-4 and the constant region of human IgG1, molecules that block T cell costimulation.

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In accordance with this aspect of the invention, the amelioration, reduction or elimination of a nonspecific immune response is enabled. The application of the method is not limited to a particular antigen-specific T cell population, but can be applied to any antigen specific T cell mediated immune response to ameliorate, reduce or eliminate the immune response. In this  
10 embodiment the mesenchymal stem cells are preferably administered locally. The mesenchymal stem cells can be either allogeneic to or autologous to the individual to whom they are being administered, preferably autologous.

Mesenchymal stem cells are normally present in minute amounts in bone marrow,  
15 however a process has been developed for isolating, purifying, and greatly replicating these cells in culture, *i.e. in vitro*. See, Caplan and Haynesworth, U.S. Patent No. 5,486,359.

The human mesenchymal stem cells can be used as hosts for foreign genes for the expression of gene products in systemic or localized targets. The human mesenchymal stem cells  
20 of the invention can be engineered (transduced or transformed or transfected) with genetic material of interest. The engineered human mesenchymal stem cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying exogenous genes therein. The culture conditions, such as temperature, pH and the like, can be those previously used with engineered human mesenchymal  
25 stem cells. See, for example, Gerson *et al.*, U.S. Patent No. 5,591,625. Mesenchymal stem cells can be treated with IFN-gamma to stimulate MHC presentation by the mesenchymal stem cells.

30

Unless otherwise stated, genetic manipulations are performed as described in Sambrook and Maniatis, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, 1989.

5           Although not limited to the treatment of autoimmune disease, the mesenchymal stem cells and method of the invention can accordingly be appropriately applied to treatment strategies requiring immunosuppressive reagents. Also contemplated is the modification of and expansion of mesenchymal stem cells *in vitro* for use in cellular immunotherapy, the *in vivo* administration of the immunosuppressive mesenchymal stem cells for treating or preventing unwanted immune  
10 responses. One aspect of the invention is the development of the mesenchymal stem cells into a vehicle for delivering inhibitory signals or antigen to target a specific cellular response, the development of vaccines with the mesenchymal stem cells modified as described herein for either target specific or systemic delivery of immunosuppression for prophylaxis and therapy of disease.

15           Typically, in the prophylaxis or treatment of disease states, the recipient is most preferably only required to undergo a single administration after which disease remission is realized on a permanent basis. Alternatively, depending upon observation of follow-up monitoring, any subsequent administration may be of greater or lesser doses. Such procedures  
20 and monitoring regimens are well known to those who are versed in the field of immune therapy, infectious disease, oncology, epidemiology and the like.

25           The dosage of the active ingredient varies within wide limits and will, of course be fitted to the individual requirements in each particular case. In general, in the case of parenteral administration, it is customary to administer from about 0.5 to about 5 million cells per kilogram of recipient body weight. The number of cells used will depend on the weight and condition of the recipient and other variables known to those of skill in the art. The cells can be administered by a route which is suitable for the particular disease state to be treated. In the case of non-specific induction of hyporesponsiveness of the immune response, mesenchymal stem cells  
30 modified with CTLA-4, for example, can be administered systemically, i.e. parenterally,

intravenously or by injection. In the case of induction of antigen specific hyporesponsiveness, the antigen-modified mesenchymal stem cells can be targeted to a particular tissue or organ, such as bone marrow.

5           The cells can be suspended in an appropriate diluent, at a concentration of from about  $5 \times 10^6$  to about  $50 \times 10^6$  cells/ ml. Suitable excipients for injection solutions are those that are biologically and physiologically compatible with the recipient, such as buffered saline solution. The composition for administration should be sterile, stable, and the like, as is appropriate for administration into an individual.

10           An advantage of the methods of the present invention over current treatment for unwanted immune responses is specificity; mesenchymal stem cells are targeted to reduce a specific immune response without adversely affecting other segments of the immune system. The amelioration, reduction or elimination of an antigen specific immune response enables the  
15 treatment of or prevention of an unwanted or abnormal immune response to a specific antigen. A further advantage is that the mesenchymal stem cells offer localized expression of a molecule of interest. Localized expression of a molecule for example which blocks co-stimulation, e.g. CTLA4-Ig, may result in reduced inflammation and fewer side effects. Examples of diseases which would be particularly suited to treatment with the mesenchymal stem cells of the present  
20 invention include thyroiditis, rheumatoid arthritis and insulin dependent diabetes mellitus. The mesenchymal stem cells can further provide a continuous supply of the molecule of interest.

25           The methods of the present invention are particularly applicable to therapy of autoimmune disease and should preferably inactivate or eliminate the response to autoantigen specifically, without compromising other aspects of the immune system.

30           The invention can be utilized for treatment of autoimmune diseases where the autoantigen mediating the disease is known. The method involves genetically engineering mesenchymal stem cells, preferably isolated from a patient's own bone marrow, to express an autoantigen in order

to induce specific immunotherapy to inactivate or eliminate abnormal immune responses, without interfering with other functions of the immune system.

The following examples further illustrate aspects of the present invention. However, they are in no way a limitation of the teachings or disclosure of the present invention as set forth herein.

## EXAMPLES

### Materials

CTLA-4 and CTLA-4Ig were expressed in the human mesenchymal stem cells transduced with the cDNA CTLA-4 sequence obtained from Gene Bank Accession # L15006. The Ig form was constructed by fusing the extracellular portion of CTLA-4 with the Fc heavy chain portion of human IgG via a flexible hinge of 4 glycines and 2 serines. Peripheral blood mononuclear cells ("PBMCs") were obtained by apheresis of the same donors of the human mesenchymal stem cells. Commercially available CTLA4-Ig was obtained from R&D Systems (Minneapolis, MN). Mouse Ly6A.2 was obtained from Dr. Ethan Shevach and cloned into a MuLV Retroviral Vector.

Tetanus toxoid specific T cell lines were prepared by stimulating PBMCs with 1µg/ml tetanus toxoid (Connaught Laboratories, Toronto, Canada) for 6 days. The cells were harvested, washed and recultured for an additional 7 days in 20 units/ml recombinant human IL2 (Proleukin) to expand tetanus toxoid specific T cells. The cells were harvested and T cells were purified by removal of B cells and monocytes using magnetic separation. The tetanus toxoid T cell line was aliquoted and frozen in liquid nitrogen until further use.

### EXAMPLE 1

#### Induction of T cell hyporesponsiveness to Tetanus Toxoid using mesenchymal stem cells.

MSCs from Donor 155 were plated in 4 T185<sup>2</sup> flasks and one flask was treated with 100U/ml IFN-gamma. After four days the MSCs were harvested (both IFN gamma treated and

untreated).  $3 \times 10^5$  MSCs were plated in each well of a 6 well tissue culture plate- 1 well with IFN-gamma treated MSCs, 1 well with untreated MSCs. IFN-gamma treated MSCs were pulsed with 50 ug/ml Tetanus toxoid overnight. All wells were washed very thoroughly with 1X Hanks Buffered Salt Solution and  $5 \times 10^6$  Tetanus toxoid specific autologous T cells (Tet T cells) (Donor 155) were added per well. The cultures were incubated for 5 days. The harvested T cells were restimulated with Tetanus toxoid (0, 0.2, 1.0 and 5.0  $\mu$ /ml) - PBMCs ( $5 \times 10^5$ /well) were used as APCs and the proliferation assay was incubated for 3 days. The cells were then pulsed with 1uCi [ $^3$ H]thymidine overnight and the assay was harvested 18 hours later.

The results illustrated in Figure 1 (control 0  $\mu$ g/ml counts subtracted as background) show a decrease (of approximately 30-40%) in the proliferative response or restimulation potential of the Tet T cells after 5 days co-culture with the IFN-gamma/Tetanus toxoid treated MSCs compared to the proliferative response of the Tet T cells co-cultured with untreated MSCs.

## EXAMPLE 2

### Blocking of T cell activation by Tetanus toxoid with CTLA-4 (membrane -bound) transduced mesenchymal stem cells

Human mesenchymal stem cells were transduced with either the full-length human CTLA-4 gene or with a mouse surface molecule (mouse Ly6A.2) as a control. Figure 2A shows the Flow Cytometry Analysis of the human mesenchymal stem cells transduced with CTLA-4. The control and the CTLA-4 transduced human mesenchymal stem cells were treated with IFN-gamma for three days to elevate MHC class II expression and were pulsed with high-dose (100ug/ml) tetanus toxoid for one day. After removal of IFN-gamma and tetanus toxoid from the cultures, a tetanus toxoid specific T cell line (TetC) was added to the human mesenchymal stem cells and the cultures were incubated for 3 days. Lymphoproliferation of the T cell line was assessed by uptake of [ $^3$ H]thymidine during the final 8 hours of the culture period.

The results, shown in Figure 3, indicated that the control transduced human mesenchymal stem cells presented tetanus toxoid to the T cells present in the PBMC preparation, and resulted in T cell proliferation (18,000 cpms), whereas the CTLA-4 transduced human mesenchymal stem cells did not induce a T cell response (200 cpms). These results demonstrated that the expression

of the CTLA-4 molecule on the human mesenchymal stem cells suppressed the T cell response.

### EXAMPLE 3

#### Blocking of T cell activation by Tetanus Toxoid with

#### CTLA4-Ig (soluble-form) transduced mesenchymal stem cells.

Human mesenchymal stem cells were transduced with either the soluble form CTLA4-Ig, which is composed of the extracellular portion of the human CTLA-4 gene fused to the constant region of human IgG1, or with a mouse surface molecule (mouse Ly6A.2) as a control. Figure 2B shows the Flow Cytometry Analysis of the mesenchymal stem cells transduced with CTLA4-Ig. To sub-confluent cultures of the control and CTLA4-Ig transduced human mesenchymal stem cells, tetanus toxoid antigen (1ug/ml) and peripheral blood mononuclear cells (PBMCs) (100,000 cells/well) were added. Lymphoproliferation of the T cells present within the PBMC preparation was assessed by uptake of [<sup>3</sup>H]thymidine during the final 8 hours of the culture period (Fig 4). Additional controls were performed where PBMCs were exposed to antigen in the presence of commercially available CTLA4-Ig protein in the absence of human mesenchymal stem cells. The results show that the CTLA4-Ig transduced human mesenchymal stem cells provided an 81% inhibition in lymphoproliferation (4,000 cpms), whereas control transduced human mesenchymal stem cells did not inhibit proliferation of T cells present in the PBMC preparation to tetanus toxoid (19,000 cpms). The inhibition (81%) in lymphoproliferation observed with human mesenchymal stem cells expressing CTLA4-Ig was greater than the level of inhibition observed with the commercially available CTLA4-Ig protein (5ug/ml) which inhibited PBMC lymphoproliferation by only 51%. Thus, the expression of the CTLA4-Ig molecule on the human mesenchymal stem cells suppressed T cell responses to levels well below those shown for commercially available CTLA4-Ig.

### What Is Claimed Is:

1. A method of inhibiting a T cell response to an antigen, comprising contacting T cells with mesenchymal stem cells modified to present the antigen, thereby inhibiting a T cell response to said antigen.

2. A method of inhibiting a T cell response to an antigen, comprising administering to a host human mesenchymal stem cells modified to present the antigen, thereby inhibiting a T cell response to said antigen.

3. The method of claim 2 wherein the antigen is an autoantigen.

4. The method of claim 3 wherein the mesenchymal cells are autologous to the host.

5. A method of inhibiting a T cell response to an antigen, comprising administering to a host human mesenchymal stem cells which express a molecule that blocks costimulation of T cells such that the T cell response to an antigen is inhibited.

6. The method of claim 5 wherein the molecule is membrane-bound.

7. The method of claim 6 wherein the molecule is CTLA-4.

8. The method of claim 5 wherein the molecule is a cell-soluble protein.

9. The method of claim 8 wherein the molecule is CTLA4-Ig fusion protein.

10. A method of inhibiting a T cell response to an antigen, comprising administering to a host human mesenchymal stem cells which are modified to express a) the specific antigen; and b) a molecule that blocks costimulation of T cells, such that the T cell response to the antigen is inhibited.



11. The method of claim 10 wherein the antigen is an autoantigen.

12. The method of claim 10 wherein the mesenchymal cells are autologous to the host.

5

13. The method of claim 10 wherein the molecule is membrane-bound.

14. The method of claim 13 wherein the molecule is CTLA-4.

10

15. The method of claim 10 wherein the molecule is a cell-soluble protein.

16. The method of claim 15 wherein the molecule is CTLA4-Ig fusion protein.

### ABSTRACT

The present invention provides a method to modulate immune responses using antigen presenting human mesenchymal stem cells to induce specific T cell anergy. The present  
5 invention also provides a method to modulate immune responses using human mesenchymal stem cells as a platform to express molecules which will induce T cell anergy.

Figure 1

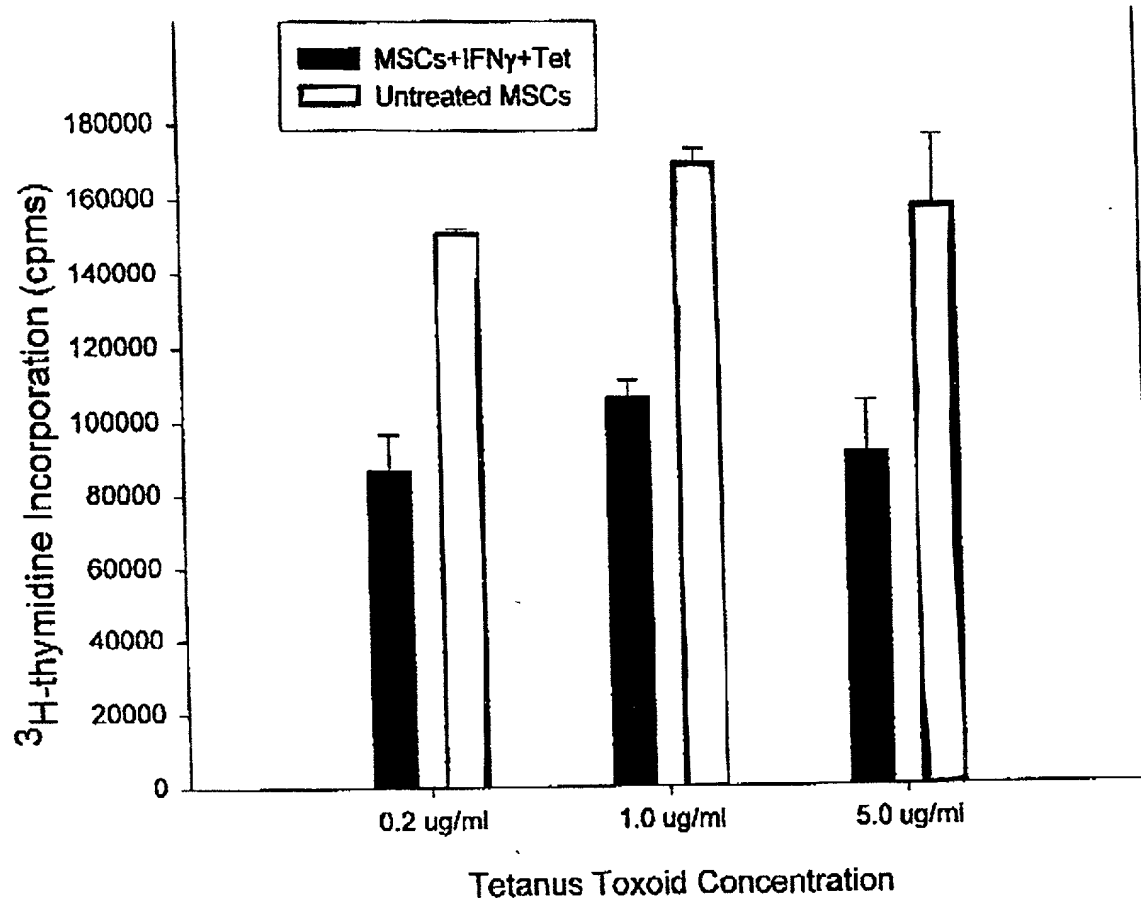


Figure 2

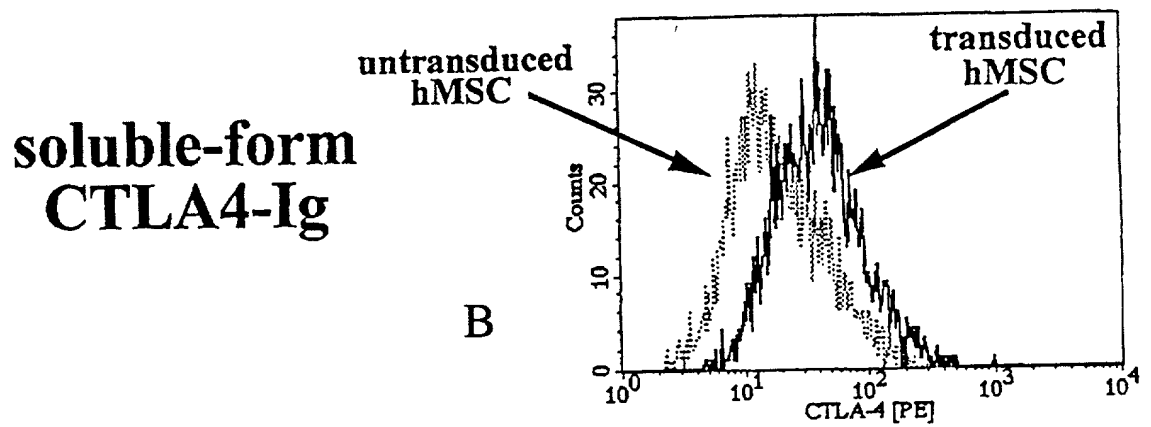
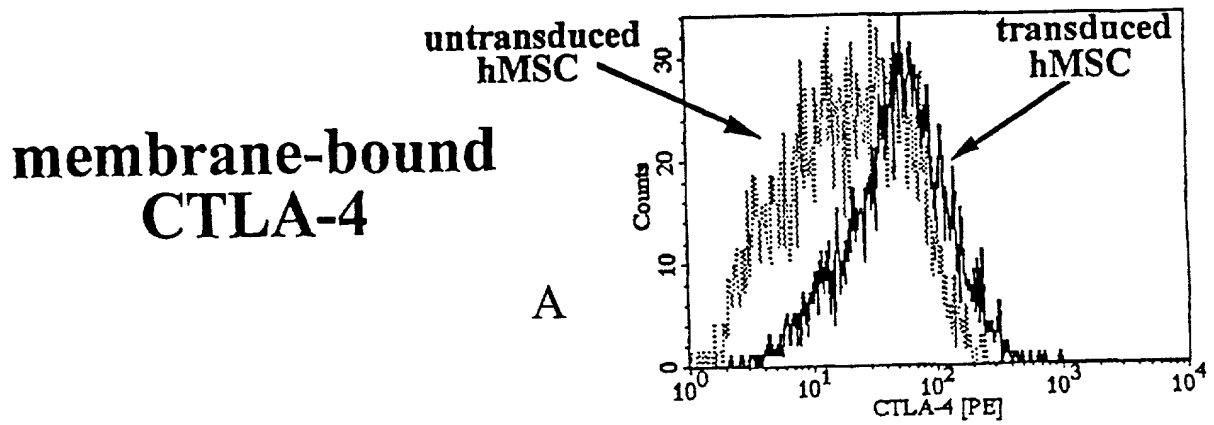


Figure 3

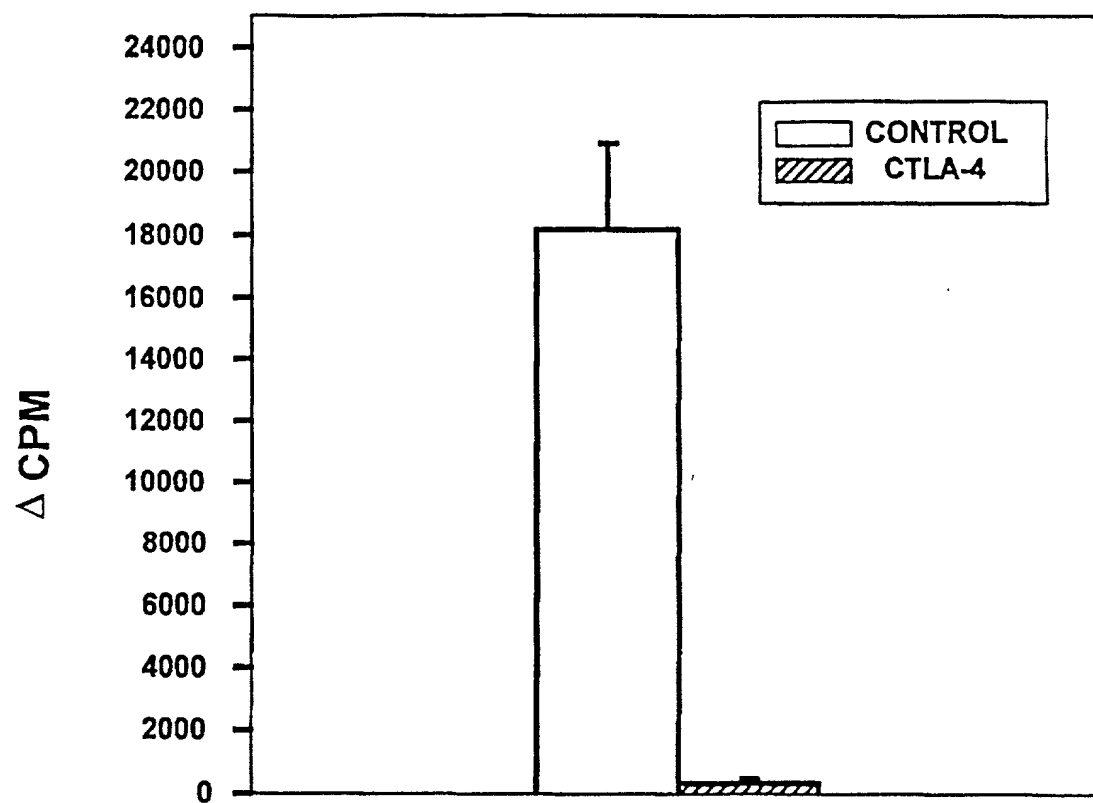
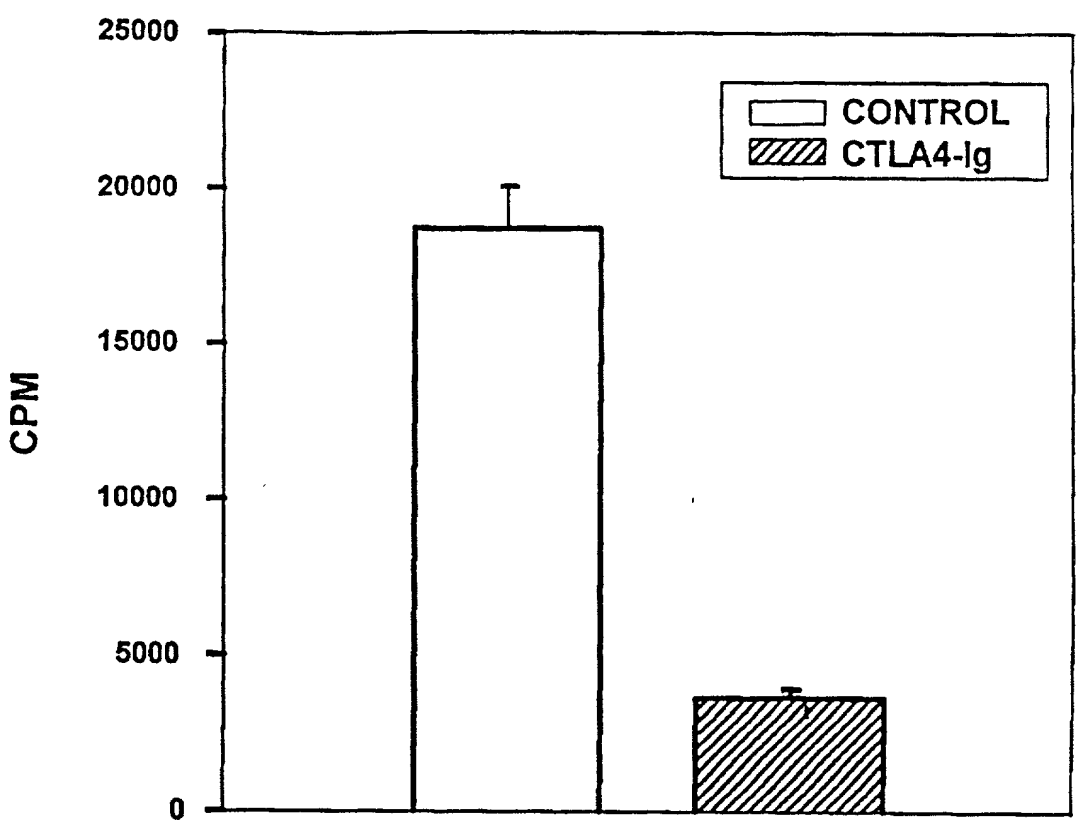


Figure 4



**DECLARATION AND POWER OF ATTORNEY**

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**MESENCHYMAL STEM CELLS AS IMMUNOSUPPRESSANTS**

the specification of which [X] is attached hereto or [ ] was filed on \_\_\_\_\_ as Application Serial No. \_\_\_\_\_ and was amended on \_\_\_\_\_ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the patentability of this application in accordance with Title 37, Code of Federal Regulations, Section 1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, Section 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed. Prior Foreign Application(s):

|          |           |                        | <u>Priority Claimed</u>  |                          |
|----------|-----------|------------------------|--------------------------|--------------------------|
| None     |           |                        | Yes                      | No                       |
| (Number) | (Country) | (Day/Month/Year Filed) | <input type="checkbox"/> | <input type="checkbox"/> |

I hereby claim the benefit under Title 35, United States Code, Section(s) 119 and/or 120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, Section 112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, Section 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

|                          |                      |                                                              |
|--------------------------|----------------------|--------------------------------------------------------------|
| <u>60/080,678</u>        | <u>April 3, 1998</u> | <u>Pending Provisional</u>                                   |
| (Application Serial No.) | (Filing Date)        | (Status – pending provisional, patented, pending, abandoned) |
| _____                    | _____                | _____                                                        |
| (Application Serial No.) | (Filing Date)        | (Status – pending provisional, patented, pending, abandoned) |

I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith: John N. Bain (Reg. No. 18,651); John G. Gilfillan, III (Reg. No. 22,746); Elliot M. Olstein (Reg. No. 24,025); Raymond J. Lillie (Reg. No. 31,778); William Squire (Reg. No. 25,378); Raina Semionow (Reg. No. 39,022); and Alan J. Grant (Reg. No. 33,389). Address correspondence and telephone calls to Raina Semionow, Esq., c/o Carella, Byrne, Bain, Gilfillan, Cecchi, Stewart & Olstein, 6 Becker Farm Road, Roseland, NJ 07068 - (973) 994-1700.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full name of sole or first inventor: Joseph D. Mosca

Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

Residence: 4201 Blue Barrow Ride, Ellicott City, MD 21042      Citizenship: USA  
Post Office Address: same

Full name of second joint inventor: : Kevin R. McIntosh

Inventor's signature: \_\_\_\_\_ Date: \_\_\_\_\_

Residence: 4225 Blue Barrow Ride, Ellicott City, MD 21042      Citizenship: USA  
Post Office Address: same